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Genetics

Two mitochondrial group I introns in a metazoan, the sea anemone *Metridium senile*: One intron contains genes for subunits 1 and 3 of NADH dehydrogenase

(animal mitochondrial DNA/intron processing/Cnidaria)

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ABSTRACT **Mitochondrial genes for cytochrome *c* oxidase subunit I (COI) and NADH dehydrogenase subunit 5 (ND5) of the sea anemone *Metridium senile* (phylum Cnidaria) each contain a group I intron. This is in contrast to the reported absence of introns in all other metazoan mtDNAs so far examined. The ND5 intron is unusual in that it ends with A and contains two genes (ND1 and ND3) encoding additional subunits of NADH dehydrogenase. Correctly excised ND5 introns are not circularized but are precisely cleaved near their 3′ ends and polyadenylylated to provide bicistronic transcripts of ND1 and ND3. COI introns, which encode a putative homing endonuclease, circularize, but in a way that retains the entire genome-encoded intron sequence (other group I introns are circularized with loss of a short segment of the intron 5′ end). Introns were detected in the COI and ND5 genes of other sea anemones, but not in the COI and ND5 genes of other cnidarians. This suggests that the sea anemone mitochondrial introns may have been acquired relatively recently.**

Organelle genes of a variety of eukaryotes contain introns classified as group I and group II (1–5). However, no introns of any kind have been reported previously in the mitochondrial genomes of multicellular animals (Metazoa). This latter finding is in keeping with the notion that metazoan mitochondrial genomes have been selected for economic use of nucleotides (6). With minor exceptions, these genomes comprise a single circular molecule that contains tightly packed genes for a constant set of 12 or 13 energy pathway-related proteins, and the 2 rRNA and 22 tRNA components of the mitochondrion's protein synthesis system (7, 8). Here we report the occurrence, contrary to expectation, of two group I introns in mitochondrial protein genes of the sea anemone *Metridium senile* (phylum Cnidaria); these introns have several unusual features.

MATERIALS AND METHODS

Animals and Nucleic Acid Isolations. All cnidarian species were obtained from Rimmon C. Fay (Pacific Biomarine Laboratories, Venice Beach, CA). Isolation of DNA and RNA from mitochondrial fractions of the *M. senile* white morph, isolation of whole cell DNA from other cnidarians, restriction enzyme digestions, cloning, DNA sequencing, and gene identifications were as described or as referred to in ref. 9.

Nucleotide Synthesis Primers. The gene locations of all primers used in the various experiments are shown in Fig. 1*B*. The following are the nucleotide sequences of primers 1–10, together with their sequence coordinates in the cytochrome *c* oxidase subunit I (COI) and NADH dehydrogenase subunit 5 (ND5) gene complexes (GenBank accession nos. U36783 and U36784, respectively). Reverse order numbers correspond to antisense sequences. A nonencoded, restriction site (RS)-containing sequence in primer 8 is bracketed: COI complex; 1, 1068–1048, 5′- TTGAGTAGTTGTAGTAAACAT; 2, 1666–1683, 5′-GGATTAAGGTACAGTCCG; 3, 1839–1819, 5′-GTGGCCAACCAACTAAACACC. ND5 complex: 4, 663–683, 5′-CGGTAAGTCTGCACAATTAGG; 5, 959–941, 5′-GGATTGTTTCAATGATCAT; 6, 1596–1573, 5′-CCTGACACTAACTCAGACTCACCC; 7, 2074–2054, 5′-AGGATCAAACCCACACTCATA; 8, 2201–2221, 5′-[GGAGATCT]GGTTATTGGACCATGATCGTG; 9, 2228–2251, 5′- GCCGTCCTAACTTTGGGTTTGGTC; 10, 2446–2427, 5′-TGCTGTAACCATAGTTGCAG.

The following are the nucleotide sequences of degenerate primers 11D–14D that are based on highly conserved amino acid sequences specified by the nucleotides of the respective gene complex. Y = C or T, R = A or G, and N = C, T, A, or G. Nonencoded, restriction site-containing sequences are bracketed. COI complex: 11D, 712–731, 5′-[CCGGATCC]TTYTGRTTYTTYGGNCAYCC; 12D, 1983–1964, 5′-[CCGGATCC]ACNACRTARTANGTRTCRTG; ND5 complex: 13D, 409–425, 5′-[GGAGATCT]TTYATYGGNTGRGARGG; 14D, 2691–2675, 5′-[CCAGATCT]GCYTTRAARAANGCRTG.

COI Splice-Site Location. To determine the nucleotide location of the COI splice site, primer 3 (0.5 pmol, 5′-end-labeled using [γ-^{32}P]ATP, and T4 polynucleotide kinase (United States Biochemical) was annealed with *M. senile* mtRNA (10 μg) by heating to 65°C, then slowly cooling to 35°C in 7 μl of 1 mM DTT and 5 units of RNasin (Promega). To the primer–template, 7 μl of supplier-provided 2× reverse transcriptase (RT) buffer was added. Each sequencing reaction (1 h at 48°C) used 2 μl of sequencing mix (a mix of dNTPs at 1.6 μM each and of one dideoxy-NTP at 0.5 μM), 2.3 μl of primer-template mix, and 0.4 unit of avian myeloblastosis virus RT (United States Biochemical). Reactions were "chased" by adding 1 μl of a mix of dNTPs at 5 mM each and 5 units of RT for 10 min at 48°C and then terminated with sequencing stop mix, and the products were fractionated in a 6% polyacrylamide sequencing gel.

Synthesis of cDNAs. cDNAs were synthesized and amplified using a Geneamp RNA PCR kit (Perkin–Elmer). An *M. senile* mitochondrial cDNA pool was generated in 20-μl reaction mixtures containing 1 μg of whole (RNase-free, DNase-pretreated) *M. senile* mtRNA, 2.5 μM random hexamers, 50 units of Moloney murine leukemia virus RT, and 1 mM each dATP, dCTP, dGTP, and dTTP. Incubation was for 10 min at 25°C, 30 min at 42°C, 5 min at 95°C, and 5 min at 5°C. PCR

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Abbreviations: COI, subunit I of cytochrome *c* oxidase; ND1, ND3, and ND5, subunits 1, 3, and 5 of respiratory chain NADH dehydrogenase; RT, reverse transcriptase; RACE, rapid amplification of cDNA ends.
Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. U36783 and U36784 for COI and ND5 gene complexes, respectively).

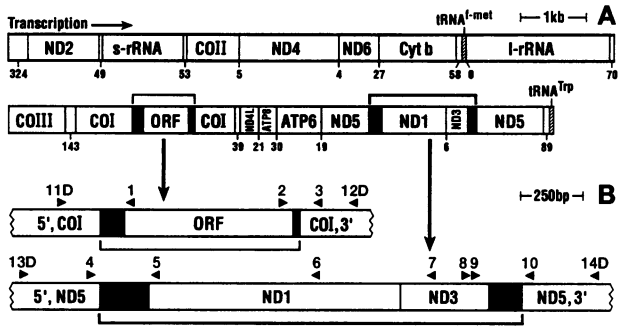

FIG. 1. (*A*) Gene map of the *M. senile* 17,433 ntp circular mtDNA molecule. There are genes for 13 proteins: Cyt b, cytochrome b; COI–COIII, subunits I–III of cytochrome *c* oxidase; ATP6 and ATP8, subunits 6 and 8 of the F_0 ATP synthase; ND1–6 and 4L, subunits 1–6 and 4L of the NADH dehydrogenase complex; the small and large subunit rRNAs (s-rRNA and l-rRNA); and two tRNAs, $tRNA^{f\text{-}Met}$ and $tRNA^{Trp}$ (hatched spaces). All genes are transcribed in the direction indicated. Numbers at gene boundaries refer to noncoding nucleotides. Introns in the COI and ND5 genes are overlined. ORF is an open reading frame in the COI intron. The ND1 and ND3 genes are within the ND5 intron. (*B*) Expanded maps of the COI and ND5 gene complexes to show the locations and directions (arrowheads) of primers 1–10 and degenerate primers 11D-14D.

amplifications (100 μl) used 25 pmol of each primer and 2.5 units of Ampli*Taq* DNA polymerase.

Rapid Amplification of cDNA Ends (RACE) Analyses. To generate a 5′ RACE pool (9, 10), the 3′ ends of mitochondrial cDNAs were poly(A)-tailed using terminal deoxynucleotidyltransferase, and complementary strands were synthesized using the *Bam*HI, *Bgl*II, and *Pst*I site (RS)-containing primer TB 17–1 (5′-CCAGATCTGGATCCTGCAGTTTTTTTTTTT-TTTTT). Double-stranded cDNAs containing the 5′ end of the ND5 intron (ND1 and ND3 transcript) were selectively PCR amplified (100-μl reaction volumes) from the 5′ RACE pool (5 μl of 100 μl) using 25 pmol each of the RS-containing primer TB 17–2 (5′-CCAGATCTGGATCCTGCAG) and primer 6 (Fig. 1*B*). The product was cleaved with *Bgl*II and *Bst*Y1 (which cleaves the intron upstream from ND1) and cloned into *Bam*HI-digested M13mp19. Insert-containing clones were identified by dot-blot analysis (11) using ^{32}P-end-labeled primer 5, and sequenced.

Two 3′ RACE pools (9, 10) were generated using oligonucleotide TB 17–1 from *M. senile* mtRNA that had been (*i*) synthetically polyadenylylated with yeast poly(A) polymerase (United States Biochemical) and (*ii*) not synthetically polyadenylylated. Selective PCR amplification of the ND5 intron cDNA 3′ ends from the 3′ RACE pools used primers TB 17–2 and primer 8 (25 pmol each). Products were cloned, identified (using ^{32}P-end-labeled primer 9), and sequenced.

Circular Intron Junction Sequences. To test for circularization of excised COI and ND5 introns, sequences were amplified from 1 μg of an *M. senile* cDNA pool using 25 pmol primers 1 and 2 (COI) or primers 6 and 9 (ND5), and an annealing temperature of 52°C (COI) or 64°C (ND5). The products were cleaved at either an *Sst*I site (nucleotide 1551 of the ND5 complex), or an *Hin*dIII site (nucleotide 1695 of the COI complex), and fragments were cloned into M13mp19 digested with *Sma*I (blunt end) and either *Hin*dIII or *Sst*I. Inserts expected to contain 5′-3′ junctions were sequenced.

Tests for COI and ND5 Introns in Various Cnidaria. Using whole cell DNA isolated from each of 11 species of Cnidaria (see text), regions of the COI and ND5 genes that contain the respective intron insertion site in *M. senile* were PCR amplified. This was done using degenerate primers 11D and 12D (COI) or 13D and 14D (ND5). The absence or presence of introns was indicated by amplification products of 429 nt (COI) and 585 nt (ND5), or about 1282 nt (COI) and 2266 nt (ND5), respectively. Amplification products were then either cloned and sequenced or cycle sequenced.

RESULTS AND DISCUSSION

MtDNA of *M. senile*, was cloned and completely sequenced (C.T.B and D.R.W, unpublished data). Analysis of the 17,443 ntp circular molecule (Fig. 1*A*) revealed genes for the same 13 proteins and 2 rRNAs characteristic of other metazoan mtDNAs. However, only two tRNAs (f-Met and Trp) are encoded, and the COI and ND5 genes each contain a group I intron (Figs. 1 and 2). The two introns have very low primary sequence similarity outside of the four highly conserved P, Q, R, and S sequences (Fig. 2). The COI intron contains an ORF, and the ND5 intron contains the ND1 and ND3 genes of which no other copies or partial copies are found in this mtDNA. We are unaware of previous reports of any introns in other metazoan mtDNAs or of group I introns in Metazoa.

The COI Intron ORF. The ORF in the COI intron extends from P6 to P9.1 and encodes a 29-kDa protein (Fig. 2 *B* and *C*). This protein includes a signature sequence, RLAGLVDGEGVF (Fig. 2*C*), of the LAGLI-DADG family of group I intron-encoded, site-specific (homing) endonucleases (4, 14, 15) that are active in transposition of group I introns into intronless alleles of the donor gene (13, 16). In *M. senile* mtDNA the inferred COI intron transposition activity may serve to reintroduce the intron, should it be lost.

Splice-Site Locations. The nucleotide locations of exon–intron boundaries in the COI and ND5 genes were predicted from comparisons of inferred amino acid sequences of the two *M. senile* genes and the corresponding genes of *Drosophila yakuba* and mouse (17, 18). Experimental confirmation of splice-site location was achieved for COI by direct dideoxynucleotide sequencing of transcripts from a downstream, antisense primer, using RT (Fig. 3*A*), and for ND5 by cycle sequencing of PCR-amplified cDNAs of transcripts containing the spliced exons (Fig. 3*B*).

Intron Excision and Processing. Excision of group I introns and exon splicing occur through two consecutive transesterification reactions (3, 19). Exogenous G attacks the 5′ splice site and covalently bonds to the intron 5′ end. The released 3′ OH of the 5′ exon attacks the phosphodiester bond that follows the universally conserved 3′ terminal G of the intron (2, 3, 12, 19), thus releasing the intron and ligating to the 3′ exon. These reactions appear to be dependent on successive binding of the exogenous G and the intron 3′ terminal G in the G-binding site of the catalytic core of the folded intron (Fig. 2*A*; refs. 3 and 19–22). Some group I introns require only exogenous G and added Mg^{2+} for correct excision-splicing of transcripts *in vitro* (3, 19, 23). However, we have not been able to detect such self-splicing for either of the *M. senile* mitochondrial introns.

The *M. senile* ND5 intron has two unusual features that might be expected to modify the splicing mechanism. The first is the presence of the ND1 and ND3 genes that are contained entirely in the loop of P8 (Fig. 2*A*). It is likely that the ND1 and ND3 genes in the ND5 intron are functional since each gene is an uninterrupted open reading frame, and there are no other ND1 or ND3 sequences in the *M. senile* mtDNA molecule. Therefore, we investigated how production of transcripts of the ND1 and ND3 genes might relate to intron processing.

The results of Northern blot analyses, using ND1-, ND3-, and ND5-specific probes and *M. senile* mtRNA are shown in Fig. 4. The main band in the ND5 lane represents an RNA of about the size expected (1800 nt) for the two ND5 exons, indicating that the majority of these exons are spliced. In each of the ND1, ND3, and ND1+ND3 lanes there is a single band representing an RNA molecule of about 1700 nt, the approximate size expected for the excised intron (1681 nt). This indicates that ND1 and ND3 transcripts are part of the same stable molecule.

The portions of the ND5 intron sequences that are linked to the ND1–ND3 transcript were determined by defining the

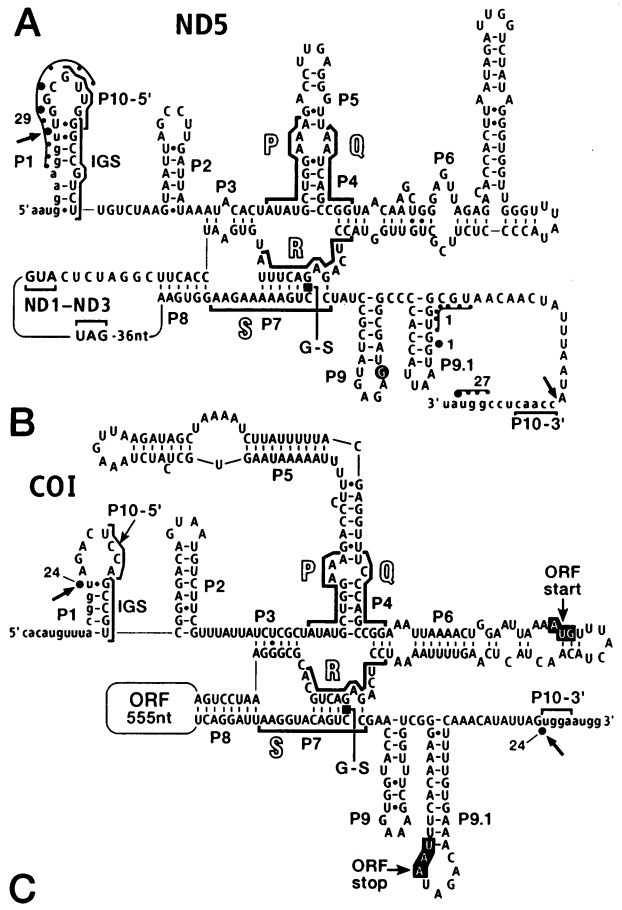

FIG. 2. (*A* and *B*) Secondary structure models for the *M. senile* ND5 and COI group I introns. Intron and exon sequences are shown as uppercase and lowercase letters, respectively. Arrows indicate splice sites. Each intron has the secondary structure potential of a group I intron (2, 12, 13) that includes helical elements P1–P10; consensus primary sequences P, Q, R, and S (hollow letters), overlined or underlined; helix P1 formed by pairing of the last 5–7 nt of the upstream exon with a sequence (internal guide sequence, IGS) near the 5′ end of the intron; the potential to pair 4- to 5-nt complementary sequences (P10–5′ and P10–3′) located between the IGS and the intron 5′ end, and the 5′ end of the downstream exon; a G·C pair (G-S, the G binding site) in helix P7. Intron circularization sites (see text) are indicated by large dots (unambiguous sites) and joined small dots [ambiguous sites (due to sequence repetition)], and accompanying numbers indicate frequencies. (*A*) The loop of P8 contains the entire ND1 and ND3 genes and the circled G in the subterminal stem is the site of polyadenylylation of the excised intron transcripts. (*B*) The start and stop codons of the ORF are boxed. The loop of P8 contains 555 nt of the ORF. (*C*) Nucleotide and inferred amino acid sequences [standard genetic code, except that TGA specifies tryptophan (9)] of the COI intron ORF. The homing endonuclease-signature sequence is boxed.

5′-end-proximal and 3′-end-proximal sequences of the transcript, using RACE analyses (Fig. 5). It was found that the 5′ end of the intron remains intact, including the terminal G

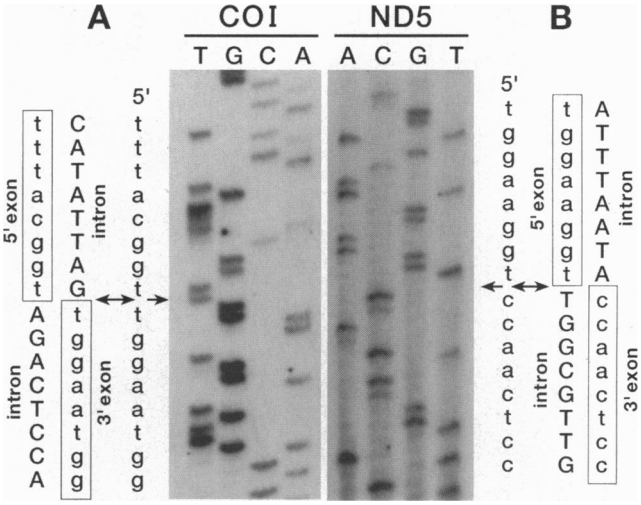

FIG. 3. Identification of the COI and ND5 splice sites. (*A*) Nucleotide sequence determined by direct dideoxynucleotide sequencing of the splice-site-containing region of the mature COI transcript using primer 3 (see Fig. 1*B*). (*B*) Nucleotide sequence of the PCR amplification product of a cDNA generated using primers 4 and 10 (see Fig. 1*B*) of the splice-site-containing region of the mature ND5 transcript, resulting from cycle sequencing, using end-labeled primer 10 (see Fig. 1*B*) and the ΔTaq cycle sequencing kit (United States Biochemical). In both *A* and *B*, genomic 5′- exon–intron and intron-3′ exon sequences, and the spliced exon sequence are shown to the left and right, respectively. Arrows indicate the splice sites.

added at the initiation of group I intron splicing (refs. 3 and 19; Fig. 5*A*). We were able to generate 3′-end-proximal sequences of ND1–ND3 transcripts from cDNAs synthesized by using a poly(T)-containing primer from mtRNAs that were or were not synthetically polyadenylylated. This indicates that ND1–ND3 transcripts are polyadenylylated *in vivo*. In five of six cloned sequences derived from natural mtRNA, a run of As follows the 43rd nucleotide from the intron 3′ end (Fig. 5*B*). The most straightforward explanation of this observation is that the 3′ 42 nt of intron transcripts are removed following orthodox intron excision at the intron–3′-exon junction. However, it is not ruled out that the observed intron transcript 3′ ends are the product of a novel processing pathway. In the one exceptional sequence, a run of As follows nucleotide 70 from the intron 3′ end, this is probably due to mispriming at the immediately downstream 5′-AAGAAAAA (Fig. 2*A*). Examination of cloned sequences derived from synthetically polya-

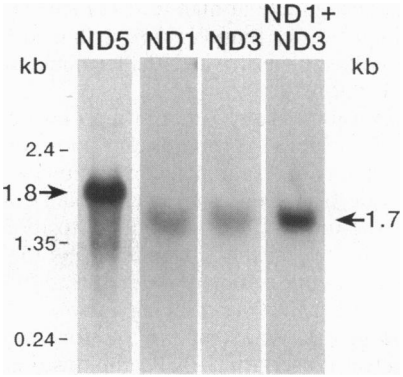

FIG. 4. Analysis of ND5, ND1, and ND3 transcripts by Northern blotting (24). Each lane contains 2 μg of *M. senile* mtRNA separated by electrophoresis through a denaturing (2.2 M formaldehyde) 1% agarose gel. The ^{32}P-end-labeled oligonucleotides used as probes were ND5, primer 10; ND1, primer 6; and ND3, primer 7 (see Fig. 1*B*). Band sizes (in kb, arrows) were determined by comparisons to a methylene blue-stained, 0.24- to 9.5-kb RNA ladder (lines; 5 μg per lane; GIBCO/BRL).

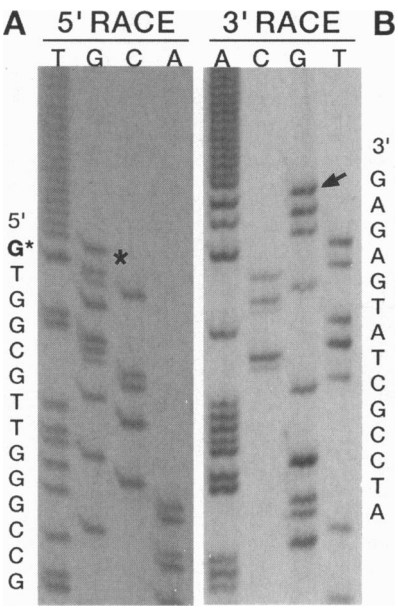

FIG. 5. Identification of the 5′ and 3′ ends of ND1–ND3 transcripts (excised ND5 introns) by 5′ and 3′ RACE analyses. (*A*) One of six identical cloned sequences of the amplified 5′-end-containing region of the excised ND5 intron transcripts. As the sequence is the transcript equivalent, the terminal reiterated nucleotide is thymidine rather than A. The sequence (left) is that expected for the 5′ end of the excised intron including the exogenous G (asterisk) added at splicing initiation. (*B*) One of five identical cloned sequences of amplified cDNAs of 3′ ends of ND1–ND3 transcripts in nonsynthetically polyadenylylated (natural) mtRNA. The sequence (*right*) indicates that polyadenylylation occurs *in vivo* following the G, 43 nt from the intron 3′ end (see Fig. 2*B*).

denylylated mtRNA yielded similar results. That is, in three cloned sequences examined, a run of As followed nucleotide 43 from the intron 3′ end in two cloned sequences and nucleotide 70 from the 3′ end in one cloned sequence.

The second unusual feature of the ND5 intron is that it ends in an A, rather than the otherwise universally conserved G (3, 19). In sequences of cDNAs that include the unspliced intron–3′-exon junction, generated by PCR using primers 10 and 9 (Fig. 1), the intron terminal nucleotide was also an A (data not shown). However, the ND5 intron has an orthodox G-binding site (a G·C pair in P7, Fig. 2*A*; see ref. 2), and clearly, the majority of ND5 transcripts from which the intron has been excised are correctly spliced (Fig. 3*B*). Also, splicing intermediates do not accumulate at high levels *in vivo* (Fig. 4), which contrasts with what was found *in vitro* with wild-type *Tetrahymena thermophila* introns in which the terminal nucleotide had been changed (20–22).

After the excision of some other group I introns, the 3′ OH of the intron 3′ terminal G can attack a phosphodiester bond near the intron 5′ end, resulting in elimination of a short linear fragment (15 or 19 nt in *T. thermophila*) and covalent circularization of the intron (3, 19). As circularization requires G binding (25), the role of the 3′-terminal A of the ND5 intron may be to deter circularization of correctly excised introns. We have tested whether, instead of being cleaved and polyadenylylated following excision, some ND5 introns might be circularized. Reverse transcription–PCR amplification was carried out using mtRNA and two primers (refs. 6 and 9; Fig. 1*B*) representing intron 5′ end-proximal and 3′ end-proximal sequences that were oriented so as to generate a product only if the intron ends were joined. Sequencing of 29 cloned products revealed that at least 27 of the circularized molecules represented resulted from joining of the ends of aberrantly excised introns. Intron circularization never involved either the 3′ terminal A or the postexcision polyadenylylation site. Among the 29 cloned sequences there were 5 different junction sequences formed by bonding between 1 of the first 5 nt of the intron or 1 of the last 3 nt of the 5′-exon, and nucleotides 8–11 of the 3′-exon (27 cases) or nucleotides in the intron terminal stem (Fig. 2*A*). All 27 of the sequences in which circularization involved nt 8–11 of the 3′-exon included the intron 3′-terminal A.

From a similar experiment for the COI intron, a reverse transcription–PCR product was also obtained. All 24 cloned sequences determined were identical (Fig. 6) and indicated that circularization resulted from joining of the intron 3′-terminal G to the intron 5′-terminal A; that is, the circular sequence contained the entire encoded intron. As we have shown that exogenous G was added to excised COI introns (data not shown), then circular COI intron sequences might have been produced by transesterification as in other group I intron circularizations (3, 19), but with removal of only the added G. Alternatively, hydrolysis of the bond following the intron 3′-end-encoded G, followed by attack by the liberated G 3′-OH at the 5′ exon/intron junction (U·A) would also be expected to generate the intron junction sequence observed. Such a circularized intron was reported as a by-product of *T. thermophila* large-subunit rRNA intron self-splicing *in vitro* (26, 27), but we are unaware of any previous report of such a circularized intron being detected *in vivo*.

Intron Distribution Among Cnidaria. The phylum Cnidaria comprises four major classes: Anthozoa [sea anemones and hard corals (subclass, Hexacorallia) and soft corals (subclass, Octocorallia)]; Hydrozoa (hydroids and hydrocorals); Scyphozoa (jellyfish); and Cubozoa (sea wasps) (28). We have tested for the presence of the COI and ND5 introns in species from 5 of the 7 different orders of Hexacorallia: *Anthopleura elegantissima*, *Tealia* sp. (order Actiniaria, to which *M. senile* belongs), *Parazoanthus* sp. (order Zoanthidea); *Astrangia lajollaensis*, *Paracyathus* sp. (order Scleractinia); *Corynactis californica* (order Corallimorpharia); and *Pachycerianthus torreyi* (order Ceriantheria); in species of two orders or Octocorallia: *Renilla kolikeri* (order Pennatulacea); and *Muricea californica* (order Gorgonacea); and in two hydrozoans, *Hydra attenuata* and *Corymorpha palma*. This was done by sizing, then (for all except *C. californica* and *H. attenuata*) sequencing the PCR products generated using pairs of degenerate primers corresponding to highly conserved sequences that bracket the *M. senile*

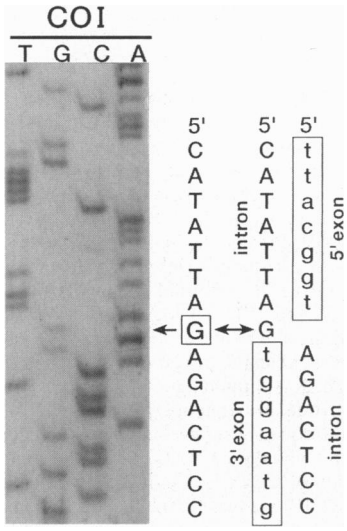

FIG. 6. The junction-containing nucleotide sequence of a circularized COI intron. Shown is one of 24 identical cloned sequences of an amplification product generated using primers 1 and 2 and mtRNA. The junction-containing sequence and genomic exon–intron sequences are written to the right. The G (boxed) at the junction is indicated by arrows.

COI and ND5 intron insertion sites. Introns were detected in both the COI and ND5 genes of the sea anemones *A. elegantissima* and *Tealia* sp., but not in any other species tested.

Concluding Remarks. The relationships of the *M. senile* COI intron ORF and COI and ND5 intron sequences to other known group I intron ORFs and group I introns remains unclear. A search of the National Center Biological Information/GenBank database (release 87) did not reveal a previously known amino acid sequence with convincing overall similarity to the COI intron ORF. The best match (8 of 12) of the *M. senile* COI intron ORF motif RLAGLVDGEGVF was to the corresponding LAGLI-DADG motif in COI intron 4 of the fungus *Saccharomyces cerevisae*. Also, the closest P, Q, R, and S sequences among known group I introns to the corresponding *M. senile* COI intron sequences are in *S. cerevisiae* COI intron 4 (29) (identities: P, 100%; Q, 90%; R, 93%; and S, 83%). The closest P, Q, R, and S sequences to those of the *M. senile* ND5 intron are in intron 4 of the *Podospora anserina* ND1 gene (30) (identities: P, 92%; Q, 60%; R, 93%; and S, 83%).

The site at which the intron is inserted in the *M. senile* COI gene is different from the sites at which introns are inserted in COI genes of other organisms. However, the *M. senile* ND5 intron insertion site is identical to those of introns in ND5 genes of the fungus *Neurospora crassa* and the bryophyte *Marchantia polymorpha* (31, 32). A lack of convincing, extensive sequence relationships among these three ND5 introns leaves open the possibility that, rather than being related by descent from a common ancestor, they may have been independently inserted at a particularly compatible, conserved site in the ND5 genes of *M. senile*, *N. crassa*, and *M. polymorpha* following their horizontal transfer from other organisms. The latter alternative is in line with the considerable accumulating evidence that group I introns can move in an infectious manner between genomes of different organisms (4, 33–35). In this regard, the finding that the COI and ND5 introns reported here are limited to actiniarians also makes it reasonable to consider if both of these introns may have been acquired by an early actiniarian mtDNA from another organism, rather than being inherited from a primordial cnidarian. Prime donor candidates would be ancestors of the endosymbiotic dinoflagellate *Zooxanthellae* and algal *Zoochlorellae* that occur in present-day *A. elegantissima* (36).

The precise processing of the *M. senile* ND5 intron, including 3′ polyadenylylation, strongly supports the notion that the two gene transcripts contained in this RNA molecule are translated into subunits 1 and 3 of the mitochondrial NADH dehydrogenase. Protein genes within group I introns, other than those concerned with intron transposition (homing endonucleases) and intron splicing (maturases), are rare: a ribosomal protein designated S5, occurs in the mitochondrial large subunit rRNA intron of *N. crassa* and other ascomycetous fungi, but details regarding expression of this protein have not been reported (37, 38). The presence of the essential ND1 and ND3 genes in the *M. senile* ND5 intron must effectively select against loss of this intron. However, how such a functional arrangement might have been established is unclear. Comparison of properties of the ND1 and ND3 genes with those of other mitochondrial protein genes of *M. senile*, including base composition, codon endings, and codon usage (C.T.B. and D.R.W., unpublished data), are consistent with an ancestral cnidarian origin of these genes. However, information regarding relative sequence identities of the ND1 and ND3 genes and other mitochondrial protein genes to the corresponding mitochondrial genes of other, non-intron-containing hexacorals will be necessary to better evaluate the possibility that the *M. senile* ND1 and ND3 genes may have been acquired together with the introns that contain them, by horizontal transfer from a noncnidarian organism.

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